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Amendment to the Specification

Please replace the paragraph bridging pages 29 and 30 with the following:

(Amended) (60/263,668) The following vectors may be designed to optimize protein expression, purification and production of proteins with the same amino acid composition as in human insulin.

a) Using tobacco plants, Eibl (1999) demonstrated, *in vivo*, the differences in translation efficiency and mRNA stability of a GUS reporter gene due to various 5' and 3' untranslated regions (UTR's). This already described systematic transcription and translation analysis can be used in a practical endeavor of insulin production. Consistent with Eibl's (1999) data for increased translation efficiency and mRNA stability, the psbA 5' UTR can be used in addition with the psbA 3' UTR already in use. The 200 bp tobacco chloroplast DNA fragment containing 5' psbA UTR may be amplified by PCR using tobacco chloroplast DNA as template. This fragment may be cloned directly in the pLD vector multiple cloning site downstream of the promoter and the aadA gene. The cloned may be exactly the same as in the psbA gene. (Update "Human Insulin") We have cloned the 5'untranslated region of the tobacco psbA gene including the promoter (5'UTR), shown in Figure 32. We performed PCR using the primers CCGTCGACGTAGAGAAGTCCGTATT SEQ. ID. NO.:4 and GCCCATGGTAAAATCTTGGTTTATTTA, which resulted in a 200 base pair product, as expected. We inserted this PCR product into a TA cloning vector. Since restriction enzyme sites were not available to subclone the 5'UTR immediately upstream of the gene coding for the CTB-proinsulin fusion protein, we used the "SOEing" PCR technique to create the DNA sequence with the 5'UTR immediately upstream of the CTB-proinsulin gene (Figure 33). The products of this PCR include both the 5'UTR (200bp) and the gene for CTB-proinsulin (600bp) as additional products as well as the desired 5'UTR CTB-proinsulin (5CP) at 800 bp. 5CP was eluded and then inserted into the TA cloning vector where DNA sequencing was performed to confirm accuracy of nucleotide sequence before it was subcloned into the pLD vector.

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PRODUCTION OF PHARMACEUTICAL PROTEINS IN TRANSGENIC PLASTIDS

BACKGROUND

(60/115,987) Research efforts have been made to synthesize high value pharmacologically active recombinant proteins in plants. Recombinant proteins such as vaccines, monoclonal antibodies, hormones, growth factors, neuropeptides, cytotoxins, serum proteins and enzymes have been expressed in nuclear transgenic plants (May et al., 1996). It has been estimated that one tobacco plant should be able to produce more recombinant protein than a 300-liter fermenter of *E. coli*. In addition, a tobacco plant produces a million seeds, thereby facilitating large-scale production. Tobacco is also an ideal choice because of its relative ease of genetic manipulation and an impending need to explore alternate uses for this hazardous crop.

(60/185,987) A primary reason for the high cost of production via fermentation is the cost of carbon source co-substances as well as maintenance of a large fermentation facility. In contrast, most estimates of plant production are a thousand-fold less expensive than fermentation. Tissue specific expression of high value proteins in leaves can enable the use of crop plants as renewable resources. Harvesting the cobs, tubers, seeds or fruits for food and feed and leaves for value added products should result in further economy with no additional investment.

(60/185,987) However, one of the major limitations in producing pharmaceutical proteins in plants is their low level of foreign protein expression, despite reports of higher level expression of enzymes and certain proteins. May et al. (1998) discuss this problem using the following examples. Although plant derived recombinant hepatitis B surface antigen was as effective as a commercial recombinant vaccine, the levels of expression in transgenic tobacco were low (0.01% of total soluble protein). Even though Norwalk virus capsid protein expressed in potatoes caused oral immunization when consumed as food (edible vaccine), expression levels were low (0.3% of total soluble protein). A synthetic gene coding for the human epidermal growth factor was expressed only up to 0.001% of total soluble protein in transgenic tobacco. Human serum albumin has been expressed only up to 0.02% of the total soluble protein in transgenic plants.

(60/185,987) Therefore, it is important to increase levels of expression of recombinant proteins in plants to exploit plant production of pharmacologically important proteins. An alternate approach is to express foreign proteins in chloroplasts of higher plant. Foreign genes (up to 10,000 copies per cell) have been incorporated into the tobacco chloroplast genome resulting in accumulation of recombinant proteins up to 30% of the total cellular protein (McBride et al., 1994).